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Cumulative effects of multiple stressors: An invasive oyster and nutrient enrichment reduce subsequent invasive barnacle recruitment

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1 **Title:** Cumulative effects of multiple stressors: an invasive oyster and nutrient enrichment
2 reduce subsequent invasive barnacle recruitment

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16 **Abstract**

17 Studies identifying interactions between biological invasions and other stressors have
18 generally focussed on quantifying their cumulative effects on mature species assemblages. In
19 benthic systems, however, early life history processes are key determinants of assemblage
20 structure and functioning. This study tested whether the presence of an invasive species
21 affected early life history processes of two common barnacle species and whether this was
22 affected by a second common stressor, nutrient enrichment. The results of a field experiment
23 identified and characterised the effects of an invasive oyster, *Crassostrea gigas*, on the early
24 life history processes of the two barnacle species under ambient and enriched nutrient
25 conditions. In the presence *C. gigas*, the invasive barnacle *Austrominius modestus*, had a
26 lower recruitment rate, however, there was no effect of the presence of *C. gigas* on native
27 barnacle, *Semibalanus balanoides*, recruitment. Nutrient enrichment also reduced the
28 recruitment rate of *A. modestus*, however, there was no evidence of synergistic or
29 antagonistic interactions between these stressors, indicating their cumulative effects were
30 additive. There was no effect of nutrient enrichment on native barnacle recruitment. Our
31 results show that the presence of an invasive oyster and nutrient enrichment altered the
32 recruitment of another non-native benthic species. These findings emphasise the importance
33 of considering early life history processes when assessing effects of multiple stressors on
34 communities.

35 1. Introduction

36 Identifying and quantifying the impacts of multiple anthropogenic stressors, such as invasive
37 species and nutrient enrichment, is a research priority in order to understand and predict
38 potential detrimental effects on ecosystems (Crain et al., 2008; Sutherland et al., 2009;
39 Strayer, 2012). Interactions between invasive species and other anthropogenic stressors can
40 lead to cumulative effects that are additive or are greater than (synergistic) or less than
41 (antagonistic) the sum of the individual effects (Folt et al., 1999; Crain et al., 2008).
42 Synergistic cumulative effects on communities are thought to be the most common (Sala and
43 Knowlton, 2006) and their occurrence has been supported by several empirical studies. For
44 example, Piazzini et al. (2005) showed a decline in percentage cover of erect algal species
45 when exposed to the invasive green algae *Caulerpa racemosa* var. *cylindracea* in increased
46 sedimentation regimes. Conversely, antagonistic interactions have also been identified, such
47 as the ability of the invasive freshwater zebra mussel, *Dreissena polymorpha*, to negate the
48 effects of nutrient enrichment on algal biomass (Dzialowski and Jessie, 2009), and the
49 presence of *Sargassum muticum*, an invasive fucoid algae, mediating the effects of nutrient
50 enrichment and warming on algal biomass (Vye et al., 2015).

51 To date, studies have focussed on the context-dependent impacts of biological invasions on
52 the diversity and functioning of mature communities (e.g. Queiros et al., 2011; Green and
53 Crowe, 2014). In benthic ecosystems, the structure and functioning of a mature community
54 can be determined by early life history processes, such as larval settlement and post-
55 settlement mortality (Connell, 1985; Gaines and Roughgarden, 1985; Hunt and Scheibling,
56 1997; Aguilera and Navarrete, 2012). Settlement, defined as the permanent attachment of
57 larvae to the substratum (Connell, 1985), is often determined by larval supply and a range of
58 settlement cues that indicate habitat suitability and resource availability, such as the presence
59 of free space and biofilm abundance (Strathmann et al., 1981; Rodriguez et al., 1993). Early

60 post-settlement mortality may be driven by predation, disturbance or physiological stress
61 (Menge and Sutherland, 1987). Both settlement and early post-settlement mortality can
62 constrain recruitment into the adult population and, therefore, are important components of
63 benthic species population dynamics (Gosselin and Qian, 1997; Delany et al., 2003; Jenkins,
64 2005). The relative importance of these early life history processes in structuring
65 communities can be context specific. Early post-settlement mortality is generally more
66 important in determining population structure in species with high recruitment rates, such as
67 barnacles (Connell, 1961a; Gosselin and Qian, 1996), whereas populations of species with a
68 lower larval supply, such as some species of corals (Hughes et al., 2000), crustaceans (Wahle
69 and Incze, 1997) and echinoderms (Balch and Scheibling, 2000), are more likely to be
70 affected by differences in settlement rates (Connell, 1961a). Invasive species, in combination
71 with other stressors, such as nutrient enrichment or warming, may drive changes in settlement
72 and post-settlement mortality by altering physical conditions, such as substratum type and
73 hydrological regimes, and biological interactions, such as competition and predation
74 (Gutierrez et al., 2003; Wilkie et al., 2013).

75 In coastal ecosystems, bivalve molluscs are common invasive species. Outside of its native
76 range, the Pacific oyster, *Crassostrea gigas*, has wide-ranging and context-dependent effects
77 on recipient communities, including driving shifts in native species assemblage structures
78 (Kochmann et al., 2008), differences in ecosystem functioning rates (Green et al., 2012), and
79 the co-introduction and facilitation of other invaders (Ruesink et al., 2005). Often the impacts
80 of *C. gigas* increase in intensity as invasion progresses and the density of the oysters
81 increases (Yokomizo et al., 2009; Green and Crowe, 2013). Although the impacts of *C. gigas*
82 on mature communities are well documented (e.g., Padilla, 2010), little is known about the
83 potential interactions between *C. gigas* and native or invasive species at early life history
84 stages (Wilkie et al., 2012).

85 *C. gigas* forms feral populations in inlets and estuaries, where eutrophication is a common
86 co-occurring stressor that can affect the impacts of biological invasions on recipient
87 communities (Lotze et al., 2006; Gennaro and Piazzzi, 2011; Vaz-Pinto et al., 2013). Thus,
88 testing whether the presence and density of *C. gigas* interacts with nutrient enrichment to
89 affect settlement and recruitment processes is a realistic scenario from which to identify the
90 context-dependent effects of invasive species. A field experiment was designed to test for the
91 separate and cumulative effects of the presence of *C. gigas* and nutrient enrichment on
92 benthic species settlement and recruitment rates. Specifically, the hypothesis tested were: (1)
93 the presence of invasive *C. gigas* and nutrient enrichment will affect the identity and
94 abundance of other benthic species settlers and recruits; (2) these putative effects will
95 interact, such that the effect of the presence of the invasive oyster on other benthic species
96 settlement and recruitment will differ between ambient and enriched nutrient conditions; (3)
97 the cumulative effects of the presence of the invasive oyster and nutrient enrichment on other
98 benthic species will be determined by oyster density.

99 **2. Material and methods**

100 *2.1. Study site*

101 The field experiment ran from February through to August 2013 at Ballygreen, a sheltered
102 intertidal sedimentary shore on the south western shore of Lough Swilly, Co. Donegal,
103 Ireland (55° 2' 31.54" N, 7° 33' 36.06" W). At this site, boulders are common and scattered
104 on sediment comprised of sandy mud, pebbles and shell fragments. Tides are semi-diurnal
105 and have a maximal range of approximately 4.5 m. The study was conducted at mid shore
106 where boulders were colonised primarily by the native barnacle *Semibalanus balanoides* and
107 the non-native barnacle *Austrominius modestus* (formerly *Elminius modestus*), the fucoid
108 algae *Fucus vesiculosus*, the honeycomb worm *Sabellaria alveolata* and the keel worm,

109 *Pomatoceros triqueter*. *Austrominius modestus* has spread rapidly since its introduction to the
110 UK and Ireland in the 1940s and may compete with native barnacle species (Bishop, 1947;
111 Crisp, 1958; Lawson et al., 2004). Lough Swilly is a relatively unpolluted estuary compared
112 to other more densely populated coastal areas of Ireland that have been classified as eutrophic
113 in assessments of water quality (Bradley et al., 2015).

114 2.2. *Experimental design and set up*

115 To quantify benthic species recruitment under manipulated conditions, forty grey opaque
116 Perspex[®] settlement plates (210 mm x 148 mm x 5 mm) were attached to the side of boulders
117 (one per boulder), which had been selected randomly along approximately 40 m x 10 m of
118 mid shore dominated by barnacles and *Fucus vesiculosus*. Grey Perspex[®] was chosen to
119 represent natural conditions based on the colour of the bedrock to minimise any differences in
120 thermal regime between the settlement plates and boulders (Lathlean and Minchinton, 2012).
121 Each plate was sanded for thirty seconds using coarse sand paper to ensure suitable rugosity
122 for settlement (Jara et al., 2006). Plates were attached to boulders at least two metres apart
123 using stainless steel screws (Stachowicz et al., 2002; Canning-Clode et al., 2008).

124 An orthogonal experimental design included two fixed factors: (i) presence of the invasive *C.*
125 *gigas* at four levels: absent, 1 individual (ind.) per plate, 4 ind. or 8 ind. (equivalent to
126 approximately 0, 32, 129, and 515 individuals per m²); and (ii) nutrient enrichment at two
127 levels: ambient conditions and nutrient enriched. Each treatment was replicated five times,
128 yielding 40 experimental units. Settlement plates were allocated randomly to treatments.
129 Non-reproductive triploid oysters (Guo and Allen, 1994) from a local aquaculture facility
130 were used to minimise effects on the feral oyster population. Juvenile oysters (spat) were
131 used in the experiment and were six months old and 36 ± 0.5 mm in length, similar to the age
132 and size of naturally settled spat at the time the experiment commenced. Spat were attached

133 to the front surface of the settlement plates using Milliput[®] epoxy putty (Dolgellau, Wales).
134 Previous work showed that there were no differences in assemblages associated with *C. gigas*
135 attached using this method compared to those with *C. gigas* attached naturally (Vye,
136 unpublished results).

137 Localised nutrient enrichment was achieved by attaching nutrient diffusers (drilled 50 ml
138 sample tubes) to each plate. Diffusers were filled with 140g of Everris Osmocote[®] Exact
139 (Geldermalsen, Netherlands) slow release fertilizer pellets (11N:11P:18K) similar to previous
140 studies (e.g., Hall et al., 2000; Minchinton and McKenzie, 2008; O'Connor and Donohue,
141 2013). Ambient treatments had diffusers filled with shell fragments to limit potential
142 experimental artefacts. Analysis of water samples from within a 15 cm radius of experimental
143 plates 8 weeks after the addition of fertiliser pellets using the same method indicated that
144 nutrient enrichment was effective (ambient total oxidised nitrogen (mean \pm S.E.): $10.54 \pm$
145 $0.81 \mu\text{m l}^{-1}$, enriched total oxidised nitrogen: $14.24 \pm 1.44 \mu\text{m l}^{-1}$, ANOVA: $F_{1, 14} = 5.014$, P
146 $= 0.042$).

147 The top surface of each plate was monitored every two to four weeks to ensure treatments
148 were maintained and photographed at eight weeks and 24 weeks (Fig. 1). Abundance of all
149 species on each plate was estimated from photos, as the new community was mono-layered
150 and this method was more accurate than estimating percentage cover using grid quadrats and
151 the point intercept method (Foster, 1991; Meese, 1992). During the experiment, the plates
152 were colonised by the native barnacle, *S. balanoides*, and the non-native barnacle, *A.*
153 *modestus*. A matrix of sediment and juvenile furoid ($< 2\text{cm}$) was present on four out of the
154 forty plates after twenty four weeks but these plates were distributed evenly among
155 treatments and this was not considered in our analysis. Total abundance of all barnacles and
156 abundance of each species were estimated using the Cell Counter plugin in ImageJ photo
157 processing software (Schneider et al., 2012). At eight weeks, barnacle settlement had

158 occurred and cyprid larvae and recently metamorphosed juvenile barnacles were present on
159 the plates. These were grouped under the term ‘settlers’ because it was not possible to
160 distinguish between cyprid and juvenile barnacles accurately (Caffey, 1985; Jenkins et al.,
161 1999; O’Riordan et al., 2004; Cruz et al., 2005; Power et al., 2006). It was also not possible
162 to identify barnacles to species level at 8 weeks and, therefore, total barnacle abundance was
163 used in analysis. At 24 weeks, barnacles were large enough to distinguish between species,
164 allowing individual estimates of the abundance and mean size of *S. balanoides* and *A.*
165 *modestus* per plate to be quantified. Estimates of percentage cover of each species relative to
166 the free space available to them were used for the analysis. *S. balanoides* individuals were
167 larger (mean \pm S.E.: 19.13 ± 0.95 mm²) than *A. modestus* (mean \pm S.E.: 15.40 ± 0.79 mm²)
168 and thus occupied a greater area of space than *S. balanoides* even when species abundances
169 were similar. Focussing on percentage cover of each species, rather than abundance, is
170 therefore more meaningful when comparing benthic recruitment rates in communities where
171 settling space is a limiting resource, such as rocky shore communities (Dayton, 1971). In
172 addition, we tested whether barnacle density differed between oysters shell and experimental
173 plates to assess whether preferential settlement on oysters occurred. An oyster was selected
174 haphazardly from plates with *C. gigas* (from treatments with 4 & 8 individuals/ plate) and
175 barnacle density on the oysters was estimated (individuals cm⁻¹) and compared to barnacle
176 density on the plates and was shown not to differ significantly ($t = 1.279$, $df = 18$, $P = 0.216$).

177 2.3. Data analysis

178 Analysis of variance (ANOVA) was used to test all hypotheses with density of *C. gigas* (four
179 levels) and nutrient enrichment (two levels) as fixed factors. Data were tested for assumptions
180 of homogeneity of variances using Levene’s test and normality was examined using Q-Q
181 plots and Shapiro-Wilk tests. Total abundance of settlers and recruits at 8 weeks and 24
182 weeks were log transformed. Percentage data were arcsine square root transformed (Sokal

183 and Rohlf, 1995). Student-Newman-Keuls tests were used to make *post-hoc* comparisons
184 among levels of significant terms. All statistical analyses were carried out in R 2.15.3 (R
185 Development Core Team, 2011).

186 3. Results

187 At eight weeks, there was no significant interaction between the presence of *C. gigas* and
188 nutrient enrichment ($F_{3,31} = 0.773$, $P = 0.518$, Fig. 2A) on the total abundance of settled
189 barnacles on the experimental plates. The presence of *C. gigas* did not affect the total
190 abundance of settled barnacles on the experimental plates significantly even at the greatest
191 oyster density level ($F_{3,31} = 1.348$, $P = 0.277$, Fig. 2A). There was no significant effect of
192 nutrient enrichment on the total abundance of settled barnacles ($F_{1,31} = 0.228$, $P = 0.636$;
193 Fig. 2A). At 24 weeks, there was no significant interaction between the presence of *C. gigas*
194 and nutrient enrichment ($F_{3,31} = 1.719$, $P = 0.183$, Fig. 2B) on total barnacle abundance.
195 There was a significantly greater total abundance of barnacles on settlement plates where *C.*
196 *gigas* was absent compared to all treatments with *C. gigas*, regardless of oyster density ($F_{3,31}$
197 $= 3.279$, $P = 0.034$, Fig. 2B and Fig. 2B(i)). However, there was no significant effect of
198 nutrient enrichment on total barnacle abundance ($F_{1,31} = 2.104$, $P = 0.183$, Fig. 2B).

199 At 24 weeks, when barnacle species could be distinguished, the native *S. balanoides*
200 constituted 26 ± 2 % (mean \pm S.E.) of the total abundance of barnacles with the remaining 74
201 ± 2 % (mean \pm S.E.) comprised of the smaller invasive barnacle *A. modestus* across all
202 treatments. Mean total percentage cover of barnacles on the plates was 15.1 ± 2.4 % (\pm S.E.).
203 There was no significant interaction between the presence of *C. gigas* and nutrient
204 enrichment ($F_{3,31} = 0.793$, $P = 0.507$, Fig. 3A) on the percentage cover of *S. balanoides*.
205 There was also no significant effect of the presence of *C. gigas* ($F_{3,31} = 1.030$, $P = 0.393$,
206 Fig. 3A), nor of nutrient enrichment ($F_{1,31} = 0.059$, $P = 0.810$, Fig. 3A) on percentage cover

207 of *S. balanoides*. There was no significant interaction between the presence of *C. gigas* and
208 nutrient enrichment ($F_{3,31} = 2.082$, $P = 0.123$) on the percentage cover of the invasive
209 barnacle *A. modestus*. Both the presence of *C. gigas* ($F_{3,31} = 3.329$, $P = 0.032$, Fig. 3B and
210 3B (i)) and nutrient enrichment ($F_{1,31} = 4.374$, $P = 0.045$, Fig. 3B and 3B (ii)) had
211 significant negative effects on the percentage cover of *A. modestus*, however, there was no
212 significant effect of increasing densities of *C. gigas* (Fig. 3B (i)).

213 **4. Discussion**

214 This study tested empirically for effects of invasive species, coupled with nutrient
215 enrichment, on the early life history processes of two species of barnacle and identified
216 negative effects of an invasive species and nutrient enrichment on invasive barnacle
217 recruitment. The effects of both factors on barnacle recruitment were independent of each
218 other indicating that the cumulative effect of both *C. gigas* presence and nutrient enrichment
219 were additive. These effects on barnacle recruitment, however, were not consistent across
220 both species, affecting an invasive but not a native species. Recruitment of the invasive
221 barnacle, *Austrominius modestus*, was lower in the presence of the invasive oyster,
222 *Crassostrea gigas*, whereas recruitment of the native species, *Semibalanus balanoides* was
223 not affected by either stressor. Furthermore, increasing the density of *C. gigas* did not
224 enhance their negative effect on recruitment of *A. modestus*, indicating that this effect was not
225 density-dependent, which shows that even at low densities the presence of an invasive species
226 can determine subsequent community dynamics. These findings also show that the effects of
227 an invasive species on other benthic species recruitment varies between different species of
228 recruits and are not determined necessarily by the presence of a secondary stressor, such as
229 nutrient enrichment, or the density of the invasive species.

230 The negative effect of the presence of *C. gigas* on recruitment of the invader *A. modestus*
231 may have been driven by reduced settlement or increased post-settlement mortality rates.
232 Differential settlement may have occurred, where all the settlers at 8 weeks, which could not
233 be identified to species level, were the native barnacle, *S. balanoides*. *Semibalanus*
234 *balanoides* showed no response to *C. gigas* or nutrient enrichment treatments at 24 weeks,
235 indicating that *S. balanoides* settlement and recruitment may not be affected by these
236 treatments. Hence, the effects seen at 24 weeks may have been a result of the subsequent
237 reduced settlement and recruitment of *A. modestus* between the two sampling events.
238 Differential settlement may occur where there are inter-species differences in larval supply or
239 settlement cues (Bohn et al., 2013). Alternatively, if differential settlement did not occur,
240 treatment effects could have been on post-settlement mortality rather than reduced settlement
241 because the effects were detected only at 24 weeks. It is possible that there was a density-
242 dependent reduction in settlers after eight weeks, as the presence of *C. gigas* reduced the free
243 space available for settlement. This is not probable, however, because of the known
244 gregarious behaviour of barnacles (Barnett and Crisp, 1979) and the relatively large amount
245 of free space (approximately 60%) remaining on the experimental plates. The similar density
246 of barnacles on settlement plates compared to *C. gigas* shells also indicates that no
247 preferential settlement occurred on the oysters that may have confounded any effects of *C.*
248 *gigas* on settlement. These findings suggest that effects of the presence of the invasive oyster
249 could have manifested at post-settlement mortality stages. Post-settlement mortality is a key
250 determinant of population dynamics in barnacles and, thus, the effects of *C. gigas* and
251 nutrient enrichment on barnacle early life history may propagate through time to impact the
252 diversity and functioning of mature benthic communities (Hunt and Scheibling, 1997; Delany
253 et al., 2003).

254 The lower total barnacle recruitment rate, primarily a result of fewer *A. modestus*, may have
255 been caused by a range of mechanisms. According to optimal foraging theory (MacArthur
256 and Pianka, 1966), the addition of *C. gigas* may represent an increase in prey resource (Pyke,
257 1984) for consumer species, such as the common shore crab *Carcinus maenas*, that may prey
258 upon both juvenile oysters and barnacles (Diederich, 2005). This may lead to enhanced
259 predation levels on the settlement plates. Alternatively, the physical presence of *C. gigas* may
260 have led to increased turbulence around the plate, increasing mortality directly owing to
261 physical disturbance (Crimaldi et al., 2002; Gutierrez et al., 2003). In addition, *C. gigas* could
262 affect mortality indirectly by mechanisms including filter feeding, which would reduce food
263 supply, and by causing differences in biofilm composition by altering hydrology (Thompson
264 et al., 2005; Neal and Yule, 2009). In each of these potential mechanisms, a density-
265 dependent effect of *C. gigas* may have been expected, however, we did not identify any
266 density-dependence in this study. This may have been because *C. gigas* covered only
267 approximately 45% of each plate at the highest density, which may have not been sufficient
268 for density effects on recruitment to become apparent (Wagner et al., 2012; Wilkie et al.,
269 2013). It has been hypothesised that the presence of invasive species may affect the
270 recruitment of other non-native species, either by increasing non-native species recruitment
271 under the invasional meltdown hypothesis (Simberloff and Holle, 1999), or by reducing non-
272 native species recruitment by increasing community invasion resistance (Elton, 1958;
273 Balmford, 1996; Levine and D'Antonio, 1999). Our results show that the recruitment rate of
274 the non-native barnacle, *A. modestus* was lower when *C. gigas* was present, which is not
275 consistent with the invasional meltdown hypothesis. Invasional meltdown occurs when there
276 are facilitative direct or indirect interspecific interactions amongst invasive species
277 (Simberloff and Holle, 1999), suggesting that in this study there were no facilitative
278 interactions between *A. modestus* and *C. gigas*.

279 Nutrient enrichment also decreased the percentage cover of *A. modestus*, which again was
280 probably driven by increasing post-settlement mortality. Direct effects of nutrient enrichment
281 on post-settlement mortality of *A. modestus* may have been caused by increases in ammonia
282 concentrations within nutrient enriched treatments, which has been shown previously to
283 affect recruitment in benthic invertebrates (Fitt and Coon, 1992), or as a result of other
284 compounds, such as potassium (Kang et al., 2004), incidentally released in nutrient enriched
285 treatments (Pawlik and Hadfield, 1990; Pawlik, 1992; Minchinton and McKenzie, 2008).
286 Nutrient enrichment may also have reduced recruitment by causing differences in the
287 abundance and composition of biofilm, an important food resource for intertidal grazers
288 (Jenkins et al., 2001; Hill and Hawkins, 2009), and therefore, increased grazing rates on the
289 settlement plates (Thompson et al., 2000). Grazing activity by the limpet, *Patella vulgata*
290 (Lewis, 1954) and the periwinkle *Littorina littorea* (Connell, 1961a; Dayton, 1971), both
291 present at the study site, have been linked to increased biological disturbance and, thus,
292 increased post-settlement mortality of newly settled cyprid larvae and juvenile barnacles
293 (Lewis, 1954; Connell, 1961b; Dayton, 1971; O'Connor et al., 2011). Our results are contrary
294 to studies in other systems that have found nutrient enrichment to increase invasion (Bertocci
295 et al., 2015; Gennaro and Piazzini, 2011), suggesting the impacts of nutrient enrichment on
296 invasive species are likely determined by the main life-history traits of the species examined.

297 Despite the predicted widespread occurrence of synergistic and antagonistic cumulative
298 impacts of multiple stressors (Sala and Knowlton, 2006; Crain et al., 2008), this study found
299 only additive cumulative effects of the presence of *C. gigas* and nutrient enrichment on *A.*
300 *modestus* recruitment. Additive effects are estimated to occur in approximately 25% of
301 multiple stressor scenarios (Crain et al., 2008) and may allow greater predictability of
302 cumulative effects where there is sufficient information describing the direct effects (e.g.
303 Chiu et al., 2008; Rius et al., 2009). The additive cumulative effects were identified over a

304 relatively short time period in this study, however, the nature of the interaction among
305 stressors may shift over longer time periods as the effects of the stressors develop (Darling
306 and Côté, 2008). In light of this, continued environmental change, such as ocean warming
307 and changes in climate variability, during community development over a longer time scale
308 may lead to more indirect and unpredictable impacts on communities and their functioning
309 (Crain et al., 2008).

310 We have shown that the additive cumulative effects of species invasion and nutrient
311 enrichment differed between recruiting species and, thus, have potential consequences for
312 population dynamics and the assemblage structure of mature communities. This study
313 highlights the importance of considering the effects of invasion, in combination with other
314 anthropogenic stressors, on processes and events across a range of life history stages in order
315 to fully comprehend multiple stressor impacts on communities. Future work should focus on
316 determining the mechanisms causing the individual and cumulative effects of invasion and
317 nutrient enrichment on recruitment using natural substrata, more complex communities and
318 over longer time periods. We should aim to identify the specific contexts at different life
319 history stages that determine interactions among multiple stressors in order to advance our
320 understanding of multiple stressor impacts.

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546

547 **Figure legends**

548 Fig. 1: Experimental plate showing settlement and recruitment of barnacles after 24 weeks.

549 Fig. 2: Mean abundance of barnacle settlers (\pm S.E.) per plate (31.1 cm²) with absence and
550 increasing densities of *C. gigas* at eight weeks (A) and 24 weeks (B). Open bars represent
551 ambient nutrient treatments and closed bars are enriched nutrient treatments. Fig. 2B (i)
552 means of *C. gigas* density treatments across ambient and nutrient enriched conditions based
553 on SNK tests. Significant differences among means are indicated by different lower case
554 letters ($P < 0.05$).

555 Fig. 3: Mean percentage cover (\pm S.E.) of *S. balanoides* (A) and *A. modestus* (B), at 24
556 weeks. Fig. 3B (i) means of *C. gigas* treatments across ambient and nutrient enriched
557 conditions and Fig. 3B (ii) means of nutrient enrichment treatments across *C. gigas*
558 treatments. Open bars and 'A' represent ambient treatments and closed bars and 'N+'
559 represent nutrient enriched treatments. Significant differences among treatments or levels of
560 treatments are indicated by lower case letters ($P < 0.05$).